

# Flow correlated percolation during vascular network formation in tumors

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A theoretical model based on the molecular interactions between a growing tumor and a dynamically evolving blood vessel network describes the transformation of the regular vasculature in normal tissues into a highly inhomogeneous tumor specific capillary network. The emerging morphology, characterized by the compartmentalization of the tumor into several regions differing in vessel density, diameter and necrosis, is in accordance with experimental data for human melanoma. Vessel collapse due to a combination of severely reduced blood flow and solid stress exerted by the tumor, leads to a correlated percolation process that is driven towards criticality by the mechanism of hydrodynamic vessel stabilization.

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Tumor vasculature, the network of blood vessels in and around a growing tumor, is in many respects different from the regular vasculature in normal tissues. Hypoxia, the lack of oxygen, that prevents a small tumor nucleus from further growth, induces the expression of various diffusible growth factors (GF) by the tumor cells that trigger a coordinated response of angiogenesis - the formation of irregular blood vessels (for a review see [1, 2]). The expected increase in microvascular density (MVD) is usually observed in the periphery of the tumor, whereas the morphology of the vasculature in the central part of the tumor is characterized by a *decreased* MVD, dilated vessels and regions of necrotic tumor tissue [3, 4]. The resulting tumor specific capillary network is very heterogeneous, composed of dense and void regions, and has a fractal dimension different from normal arteriovenous or normal capillary networks [5].

Although on the molecular level the main actors in the angiogenic game are rapidly identified, the physical principles that determine the global morphology of the vascular network in tumor tissues are not known. Since for instance MVD is used as a diagnostic tool in cancer therapy [6] a quantitative understanding of the mechanism that leads to the compartmentalization of the tumor vasculature into various regions differing substantially in vessel density appears mandatory. Moreover, scale-invariant aspects like fractal dimension, are used as hints towards the nature of the growth process underlying the formation of the tumor vasculature [7]. In this Letter we propose a theoretical model for the evolution of tumor vasculature that illuminates the physical principles leading to its global morphology. The experimentally observed increase in MVD at the tumor perimeter and periphery and decrease in MVD and vessel dilation in the tumor center in human melanoma [4] appears also as the general scenario in the theoretical model that we discuss. Furthermore, we will argue that vessel collapses in the interior of the tumor lead to a percolation process which is driven towards criticality, the percolation threshold, via a mechanism of vessel stabilization by increased blood flow in the remaining vessels.

Guided by a two-dimensional cellular automaton model that two of us developed recently [8] we consider the tumor-vessel system as a dynamically evolving network or graph interacting with a tumor growth process (inspired by the Eden model [9]). The interaction takes place via two concentration fields: the oxygen originating in the vessel network, and the growth factor originating in the tumor cells (TC). A hydrodynamic flow is imprinted on the vessel network that emits oxygen. TC's proliferate/die when the local oxygen concentration is high/low. Vessels (edges) emerge when the local GF concentration is high enough, and they vanish (collapse) stochastically inside the tumor, when the hydrodynamic shear force acting on the vessel walls is too low. The biological and pathophysiological motivation for the details of the model definition to follow is discussed in [8].

To be specific, we describe the topology of the vessel network by a graph  $G = (V, E)$ , and identify each edge  $e \in E$  with a vessel, and each node  $v \in V$  with a vessel junction, where more than two vessels merge. Here we restrict to capillary networks and do not discriminate between arteries and veins. The network  $G$  is embedded in the three-dimensional Euclidean space  $R^3$  and restricted to the cube  $Z \subset R^3$  of volume  $\mathcal{L}^3$ . This cube is discretized into  $L^3 = (\mathcal{L}/a)^3$  unit cells, where a dimensionless vector  $\mathbf{r} = x\mathbf{i} + y\mathbf{j} + z\mathbf{k}$  with  $x, y, z = 0, 1, 2, \dots, L-1$  denotes each unit cell. The microscopic length scale is chosen to be  $a = 10\mu m$ , the typical size of the endothelial cell (EC) and TC.

For computational convenience we restrict the edges to run only parallel to the three coordinate axes and identify an edge with the string of unit cells of  $Z$  that it covers: Let  $\mathbf{r}_s(e)$  and  $\mathbf{r}_t(e)$  be the two end-points of an edge  $e \in E$  and  $\ell(e) = |\mathbf{r}_t(e) - \mathbf{r}_s(e)| = n(e) - 1$  the length of the vessel, then

$$e = \{\mathbf{r} = \mathbf{r}(\mathbf{r}_s(e), \mathbf{r}_t(e), \zeta) \mid \zeta = 0, 1, 2, \dots, \ell(e)\} \quad (1)$$

with  $\mathbf{r}(\mathbf{r}_s, \mathbf{r}_t, \zeta) \equiv \mathbf{r}_s + \zeta(\mathbf{r}_t - \mathbf{r}_s)/\ell(e)$ . Note that  $V = \{\mathbf{r}_s(e) \mid e \in E\} \cup \{\mathbf{r}_t(e) \mid e \in E\}$ .

The tumor is represented by the set  $T$  of points that are occupied by tumor cells:  $T = \{\mathbf{r} \mid \text{A TC exists at } \mathbf{r}\}$ . The ves-

sel network  $G$  is the source of an oxygen concentration field  $O_2(\mathbf{r})$  and the tumor  $T$  is the source of a growth factor concentration field  $GF(\mathbf{r})$ :

$$GF(\mathbf{r}) = \sum_{\mathbf{r}' \in T} h_{R_{gf}}(|\mathbf{r} - \mathbf{r}'|),$$

$$O_2(\mathbf{r}) = \sum_{\mathbf{r}' \in E} h_{R_{oxy}}(|\mathbf{r} - \mathbf{r}'|).$$

$R_{gf}$  and  $R_{oxy}$  are the growth factor and oxygen diffusion radii, respectively, and for simplicity we choose a piecewise linear and normalized form for the contribution  $h_R(r)$  of each tumor cell / vessel segment,  $h_R(r) = (1 - r/R)/(\pi R^3/3)$  for  $r < R$  and  $h_R(r) = 0$  for  $r \geq R$ , satisfying  $\int_0^\infty dr h_R(r) 4\pi R^2 = 1$ .

Each edge  $e$  represents a tubular vessel of diameter  $d(e)$ , through which a hydrodynamic blood flow of magnitude  $q(e)$ , exerting a shear force  $f(e)$  upon the vessel walls, can pass. The flow is assumed to be an incompressible laminar stationary flow for which  $q(e)$  and  $f(e)$  are calculated by Poiseuille's law:

$$q(e) = d^4(e) \nabla P(e), \quad \text{and} \quad f(e) = d(e) \nabla P(e), \quad (2)$$

where the pressure gradient in the vessel  $e$  is defined via  $\nabla P(e) = |P(\mathbf{r}_i(e)) - P(\mathbf{r}_s(e))|$  and the pressure  $P(\mathbf{r})$  in the nodes (vessel junctions) of the network is computed using Kirchhoff's law. The boundary condition for the blood pressure  $P(\mathbf{r})$  is static and chosen in such a way that blood flow as well as shear force in the original network is homogeneous:  $P(\mathbf{r} = (x, y, z)) = 1.5|x + y + z|/[3(L - 1)]$  at the boundary  $\partial Z = \{\mathbf{r} = (x, y, z) | x = 0 \text{ or } y = 0 \text{ or } z = 0\}$ .

Initial configuration: The original tissue is regularly vascularized with a homogeneous capillary network of given MVD that is fixed by inter-vessel distance  $\delta$ :  $E = E_0 = \{e | \mathbf{r}_s(e) = \delta(n_1, n_2, n_3), \mathbf{r}_t(e) = \mathbf{r}_s(e) + \delta\mathbf{i}, \delta\mathbf{j}, \text{ or } \delta\mathbf{k}, n_1, n_2, n_3 = 0, 1, \dots, N - 1\}$  with  $N = \lfloor L/\delta \rfloor + 1$ . The number of nodes  $n(V)$  is  $N^3$ , that of edges  $n(E)$  is  $3(N - 1)N^2$ , and  $n(e) = \delta + 1$ . For each edge  $e$ ,  $d(e) = 1(10\mu\text{m})$ ,  $q(e) = q_0$ , and  $f(e) = f_0$  with  $q_0 = f_0 = 0.5/L$  from Eq. (2). A tumor nucleus containing  $N_{TC}$  tumor cells and grown using the Eden rule [9] starting with a seed at the system center  $\mathbf{r}_c = (L/2, L/2, L/2)$  defines the set of tumor cells  $T$  at time  $t = 0$ .  $t_{uo}(\mathbf{r}) = 0$  for all  $\mathbf{r} \in T$ , representing the time spent in hypoxia. Starting with this initial configuration the following computations are performed sequentially in each time step of duration  $\Delta t = 1\text{h}$ .

TC proliferation: Proliferation of tumor cells is possible at tumor surface sites  $\mathbf{r} \in S = \{\mathbf{r} | \mathbf{r} \notin T, \mathbf{r}' \in T, |\mathbf{r}' - \mathbf{r}| = 1\}$  if the local  $O_2$  concentration is sufficiently large:  $T \rightarrow T \cup \{\mathbf{r}\}$  with probability  $\Delta t/t_{TC}$  if  $O_2(\mathbf{r}) > c_{oxy}$ .

Vessel growth: New vessels (edges in  $G$ ) can be grown (added to  $G$ ) in regions, where the local GF concentration is sufficiently large: *i*) Randomly choose  $e_1 \in E$  and  $\mathbf{r}_1 \in e_1$ . *ii*) If there is  $\mathbf{r}_2 \in e_2$  for a certain  $e_2 \neq e_1$  so that  $\mathbf{r}_2 - \mathbf{r}_1 \parallel \mathbf{i}$  or  $\mathbf{j}$  or  $\mathbf{k}$ ,  $2 \leq |\mathbf{r}_2 - \mathbf{r}_1| \leq \ell_{\max}$ ,  $GF(\mathbf{r}(\mathbf{r}_1, \mathbf{r}_2, \zeta)) > c_{gf}$  for all  $\zeta = 0, 1, \dots, |\mathbf{r}_2 - \mathbf{r}_1|$ , and  $\mathbf{r}(\mathbf{r}_1, \mathbf{r}_2, \zeta) \notin e$  for any  $e \in E$  with  $\zeta = 1, \dots, |\mathbf{r}_2 - \mathbf{r}_1| - 1$ , then  $E \rightarrow E \cup \{e_{\text{new}}\}$  with  $e_{\text{new}} = \{\mathbf{r} | \mathbf{r} = \mathbf{r}(\mathbf{r}_1, \mathbf{r}_2, \zeta), \zeta = 0, 1, 2, \dots, |\mathbf{r}_2 - \mathbf{r}_1|\}$ . Repeat *i*) and *ii*)

$\delta n(E)\Delta t/t_{EC}$  times, since the number of potential sprouting events should be proportional to the total length of the vessels in the network.

Vessel dilatation: Within the tumor no new vessels can occur because of the lack of space, but vessels increase their diameter due to proliferation of EC's in the vessel walls if the local GF concentration is sufficiently large: For all  $e \in E$ ,  $d(e) \rightarrow d(e) + \sum_{\mathbf{r} \in e} \Theta_{\text{step}}(GF(\mathbf{r}) - c_{gf})/(\ell(e) + 1)/2\pi$  with probability  $\Delta t/t_{EC}$  if  $d(e) < d_{\max}$ . Here  $\Theta_{\text{step}}(x) = 1$  for  $x \geq 0$  and  $\Theta_{\text{step}}(x) = 0$  for  $x < 0$ .

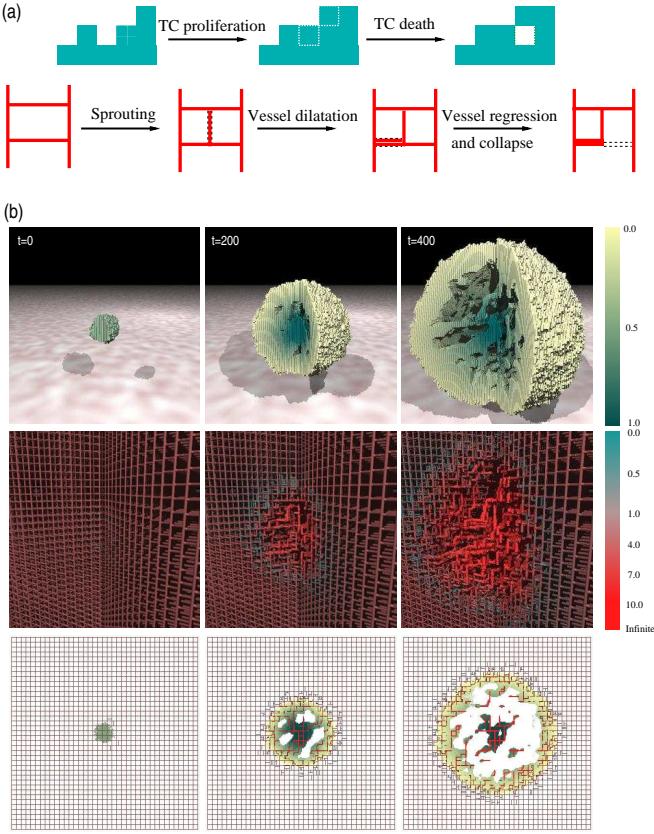
Vessel regression and collapse: Weakly perfused vessels can collapse inside the tumor due to the solid stress exerted by the tumor: Compute  $P(\mathbf{r})$  for all  $\mathbf{r} \in V$  and then  $f(e)$  and  $q(e)$  according to Eq. (2). Vessels that are cut from the blood circulation ( $q(e) = 0$ ) are instantaneously removed. For all other  $e \in E$  we set  $E \rightarrow E - \{e\}$  with probability  $\Delta t/t_{\text{collapse}}$  if  $f(e)/f_0 < \eta_c$  (i.e. a weak shear force), and  $n(\{\mathbf{r} | \mathbf{r} \in T, |\mathbf{r} - \mathbf{r}'| \leq 1 \text{ for } \mathbf{r}' \in e\}) \geq 0.8\ell(e)$  (i.e. a large density of TCs around the vessel).

TC death: TC's that are under-oxygenated longer than a time  $t_{\max}$  will die: For all  $\mathbf{r} \in T$ ,  $t_{uo}(\mathbf{r}) \rightarrow t_{uo}(\mathbf{r}) + 1$  if  $O_2(\mathbf{r}) \leq c_{oxy}$ . If  $t_{uo}(\mathbf{r}) > t_{\max}$ ,  $T \rightarrow T - \{\mathbf{r}\}$  with probability  $1/2$ .

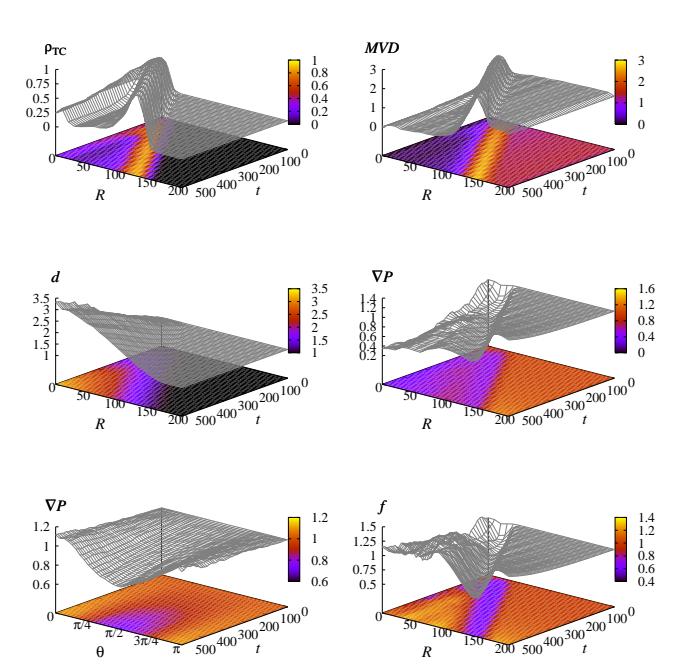
A schematic illustration of these procedures is shown in Fig. 1. We have simulated the model using various parameter values, but here we restrict ourselves to the discussion of one typical parameter set, which is partly guided by data for human melanoma [4]. The original MVD is set by  $\delta = 100\mu\text{m}$  and the oxygen diffusion radius  $R_{oxy} = 100\mu\text{m}$ , yielding an average  $O_2$  concentration of  $\bar{O}_2 \approx 0.03$ . The oxygen threshold for TC proliferation and for cell death due to hypoxia is  $c_{oxy} = 0.01$ , i.e. clearly below  $\bar{O}_2$ . TC and EC proliferation time are  $t_{TC} = 10\text{h}$  and  $t_{EC} = 40\text{h}$ , respectively, maximum TC survival time in hypoxia  $t_{\max} = 20\text{h}$ . The GF diffusion radius is  $R_{gf} = 200\mu\text{m}$ , the GF threshold  $c_{gf} = 0.001$ . The maximum vessel diameter is  $d_{\max} = 35\mu\text{m}$ , maximum sprout migration distance  $\ell_{\max} = 100\mu\text{m}$ , critical shear force  $\eta_c = 0.5$  and vessel collapse rate  $1/t_{\text{collapse}} = 1/50\text{h}$ . The initial tumor size  $N_{TC} = 27000$  (i.e. an initial tumor diameter of ca.  $0.6\text{mm}$ ).

An example for the time evolution of the tumor/vessel system in this model is shown in Fig. 1. Starting from a regular vessel network the MVD in the peritumoral region is increased due to the supply of GFs from the tumor, as can best be seen in the snapshots of an equatorial cross section through the tumor center (last row of Fig. 1). Once the tumor grows over this highly vascularized region, vessels start to collapse, by which the MVD in the interior of the tumor is continuously decreased until only a few thick vessels, surrounded by cuffs of TCs remain. Due to the reduced MVD in the tumor center regions become hypoxic and TCs will die leaving large necrotic regions. This compartmentalization of the tumor into different shells that can be discriminated by MVD, vessel diameter and necrosis is also observed in real tumors [4].

Fig. 1 shows that the original vascular network, consisting of capillaries of equal diameter arranged in a regular grid with a given MVD that guarantees homogeneous distribution of  $O_2$  and a constant shear stress in all vessels, is dynamically trans-



formed into a compartmentalized network with irregularly arranged dilated vessels and characterized by an inhomogeneous MVD and  $O_2$  distribution. This remodeling is strongly correlated with the blood flow pattern: When new vessels are generated, they share the blood flow with their parents, which causes all of them to have weaker shear forces, subject to potential vessel collapse. On the other hand, when such critical vessels are indeed removed, the blood flow has again to be redirected into the remaining vessels, leading to an increase of the shear forces in the involved vessels. At the same time, surviving vessels may increase their diameters, also resulting in higher shear forces. The ratio  $t_{EC}/t_{collapse}$  basically controls this flow-correlated remodeling process consisting of generation / dilatation and collapse and also affects the tumor volume or necrosis: Necrosis dominates the tumor tissue in lack



of oxygen supply for a larger value of the ratio while the total necrotic region shrinks for a smaller value.

The dynamical evolution described thus far can be analyzed quantitatively by studying the following quantities: The radial tumor density  $p_{TC}(R) = n(T_R)/n(Z_R)$ , vessel density  $MVD(R) = n(E_R)/(4\pi R^2)$ , vessel diameter  $d(R) = \sum_{e \in E_R} d(e)/\sum_{e \in E_R}$ , pressure gradient  $\nabla P(R) = \sum_{e \in E_R} \nabla P(e)/\sum_{e \in E_R}$ , and  $\nabla P(\theta) = \sum_{e \in E_\theta} \nabla P(e)/\sum_{e \in E_\theta}$ , and shear force  $f(R) = \sum_{e \in E_R} f(e)/\sum_{e \in E_R}$ . Here we have used  $Z_R = \{\mathbf{r} | R \leq |\mathbf{r} - \mathbf{r}_c| < R + \Delta R\}$ ,  $E_R = \{e \in E | R \leq |(\mathbf{r}_s(e) + \mathbf{r}_t(e))/2 - \mathbf{r}_c| < R + \Delta R\}$ ,  $E_\theta = \{e \in E | \theta \leq \cos^{-1} \frac{(\mathbf{r}_s(e) - \mathbf{r}_c) \cdot \mathbf{r}_c}{|\mathbf{r}_c - \mathbf{r}_s(e)|} < \theta + \Delta \theta\}$ , and  $T_R = T \cap Z_R$ .

As seen in Fig. 2, the peak of  $MVD(R)$  is in accordance with the tumor boundary  $R_{TC}(t)$ . The tumor grows approximately spherically with  $R_{TC}(t) - R_{TC}(0) \simeq 2t/t_{TC}$ , where the factor 2 is typical for the Eden growth. In the tumor center,  $MVD(R)$  and  $p_{TC}(R)$  are both very low. Their shape similarity is due to continuous interactions between the tumor and the vessel network. Vessels that have long been exposed to GF produced by TCs have large diameters:  $d(R)$  increases linearly from 1 at  $R \simeq R_{TC} + R_{gf}$  to  $d_{max}$  at the tumor center. Such a characteristic vessel morphology is also in a quantitative agreement with experimental data from the human melanoma [4].

The blood pressure gradient in the tumor center is up to 50% lower than in normal vessels which is, from hydrodynamic considerations, an immediate consequence of the in-

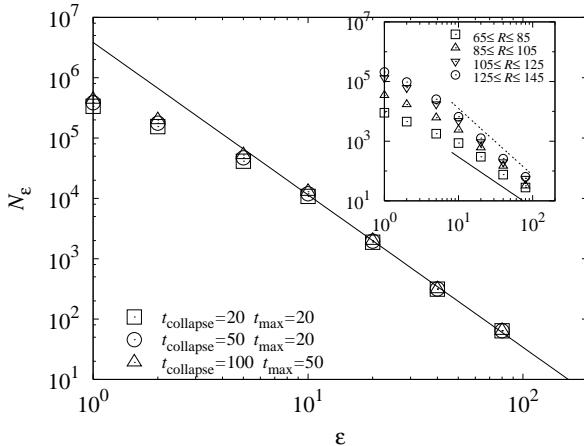


FIG. 3: Plots of  $N_\epsilon$  of Eq. (3) in the vessel networks at  $t = 400$  for different values of  $t_{\text{collapse}}$  and  $t_{\text{max}}$  with other parameters taking the typical values. The tumor and the peritumoral region extend up to  $R \lesssim 145$ , for which  $N_\epsilon$  is computed. In all cases, the data are fitted as  $N_\epsilon \sim \epsilon^{-2.52(5)}$ . The solid line has a slope  $-2.52$ . The inset shows  $N_\epsilon$  measured in different shells of the same thickness 20 for  $t_{\text{collapse}} = 20$  and  $t_{\text{max}} = 20$ . The slopes of the upper dashed line and lower solid line are  $-2.24$  and  $-1.68$ , respectively.

crease MVD in the peritumoral region. Moreover, the pressure gradient is lowest in the direction orthogonal to the global flow ( $\theta = \pi/2$ ), since for a given network the flow tends to use shortest paths from source to sink. Finally, the shear force acting on each vessel wall depends on the vessel diameter and the pressure gradient. The sharp decrease of  $\nabla P$  in the peritumoral region is inherited to  $f(R)$  while the increased vessel diameter in the tumor center gives rise to large shear forces in large vessels.

The geometrical features of the emerging tumor vasculature in our model are obviously very different from the original, regular capillary network: It consists of a combination of dense and void regions that might possess fractal properties. We used the box-counting method to determine the fractal dimension  $D_f$  as

$$D_f = -\lim_{\epsilon \rightarrow 0} \frac{\ln N_\epsilon}{\ln \epsilon}, \quad (3)$$

where  $N_\epsilon$  is the number of boxes of volume  $\epsilon^3$  necessary to cover the tumor vessel network, that is defined to lie within the outer limit of the peritumoral region. The plot of  $N_\epsilon$  versus  $\epsilon$  shown in Fig. 3 yields  $D_f = 2.52(5)$ , in agreement with the value for the critical percolation cluster in the random bond-percolation process in three dimension [10]. We checked that we obtain the same value for a wide range of parameter values.

From this observation we can conclude that the basic mechanism responsible for the fractal properties of the tumor vasculature in our model is the stochastic removal of vessels via vessel collapse and regression. In conventional percolation a critical cluster only emerges if the edge concentration is fixed to be exactly at the percolation threshold. In our model this

fine tuning is not necessary: the dynamically evolving network drives itself into this critical state since the removal of vessels is correlated with the blood flow. The collapse of critical vessels stabilizes the remaining ones due to an increase in blood flow, as shown in our quantitative analysis.

It has been suggested [5] that the origin of the fractal architecture of tumor vasculature might be due to an underlying invasion percolation process [11] due to inhomogeneities in the growth supporting matrix. In view of the theoretical model we have presented, which does not involve any such matrix-inhomogeneities, we propose that it is rather the flow correlated percolation process due to collapsing vessels inside the tumor that determines the fractal properties of the tumor vasculature. A commonly accepted view, at least for a large class of tumors like melanoma, also shared by our theoretical approach, is that neo-vascularization mainly occurs at the tumor perimeter and a drastic reduction of vessel density occurs in the interior of the tumor. In such a scenario it appears unlikely that the fractal properties attained during growth in the periphery, independent of having characteristics of invasion percolation or not, survive the random dilution process in the tumor center. Thus a theoretical model that does not take into account vessel collapse can possibly explain the observed fractal dimension of the vessel network in tumors where the central MVD is *not* drastically reduced but fails to do so in networks where it is.

To conclude we have introduced a theoretical model for a dynamically evolving, three-dimensional vessel network interacting with a growing tumor, which is guided by experimental data for human melanoma. The emerging network morphology agrees well with those data and we find that the network is remodeled from a regular into a fractal structure with characteristics of random percolation. This suggests also for a large class of real solid tumor with decreased central MVD that the basic mechanism leading to the fractal features of the tumor vasculature is the mechanism of stochastic vessel collapse inside the tumor.

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